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Title:

Bioacoustic and biophysical analysis of a newly described highly transparent genus of predatory katydids from the Andean cloud forest (Orthoptera: Tettigoniidae: Meconematinae: Phlugidini)

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Abstract

Transparency is a greatly advantageous form of camouflage, allowing species to passively avoid detection regardless of the properties of the surface which they occupy. However, it is uncommon and poorly understood in terrestrial species. In one tribe of predacious katydids (Phlugidini), transparency is paired with highly ultrasonic communication for increased predator evasion, yet little is known about the singing capabilities of these species, with only one genus of Phlugidini acoustically well described to date. Here, we describe *Speculophlugis hishquten* new genus and species of highly transparent crystal katydid species from the Andean cloud forest, discussing the potential use of this species for non-invasive studies of internal anatomy, and analysing its ultrasonic call. Using laser Doppler vibrometry and light microscopy techniques, we found the transparency of the cuticle around the hearing apparatus to be 85-87 % at the wavelength of the laser beam (633 nm), making *S. hishquten* a candidate for the highest recorded cuticle transparency of any insect. The male song has a fundamental frequency of 50 kHz, matching both the ultrasonic call range and rapid call structure of other Phlugidini species. However, the extent of ultrasonic communication and the level of transparency across the Phlugidini tribe requires more attention.

Keywords: Camouflage, bushcricket, ultrasonic, Stridulatory file, taxonomy, neotropical

Introduction

Defined as the concealment of the body and prevention of detection (Stevens and Merilaita 2008), camouflage allows individuals to avoid perception by other organisms; reducing mortality and increasing predation success (Merilaita et al. 2017). The most frequently observed camouflage strategies include masquerade; the mimicry of organisms or objects within an environment (Browner et al. 1964; Merilaita et al. 2017), and background matching; the evolution of pigments and patterns to disguise organismal outlines (Stevens and Merilaita 2008; Merilaita et al. 2017). Examples of such strategies in nature are plentiful, but perhaps the greatest evolutionary marvel of camouflage is the strategy of transparency.

The primary advantage of transparency is passive crypsis; reducing the reflectiveness of biological surfaces to avoid immediate detection (Johnsen 2001). Organisms with poor vision exploit passive crypsis in order to increase their proximity to prey before detection, and to remain undetected regardless of the surface properties of the substrate which they occupy (Greene 1983; Johnsen 2001). Additionally, organisms may combine transparency with other physiological adaptations for unique hunting niches. For example, some siphonophores accompany transparency of the body with small pigmented regions, which act as lures for prey by mimicking larval fish (Purcell 1980).

Despite these apparent principal advantages of transparency, it is not a greatly recorded trait in insects or other terrestrial species and is most widely reported in pelagic species such as deep ocean Cnidarians and Ctenophores (Johnsen 2000, 2001), and marine shrimps (Carvalho et al. 2006; Bhandiwad and Johnsen 2011). This is not to say however that we do not observe any terrestrial transparent species. For example, frogs of the family Centrolenidae (Guayasamin et al. 2008) and clearwing Lepidopterans of the family Sesiidae both show unique forms of transparent camouflage. However, the paragons of terrestrial transparency

are the stealthy predatory katydids of the tribe Phlugidini (Orthoptera: Tettigoniidae: Meconematinae). With 13 Australasian species of 3 genera (Otte 1997; Rentz, 2010; Cigliano et al. 2019), 58 Neotropical species of 9 genera (Nickle 2003; Cigliano et al. 2019), and 25 Asian species of 3 genera (Tan 2011; Cigliano et al. 2019), this widespread group is morphologically well described (Nickle 2003, 2005; Tan et al. 2019), yet their translucent and even transparent cuticles, which could hold key explanations for the evolution of terrestrial transparency, has not yet been investigated. In addition, the high transparency of the cuticle could allow for multiple non-invasive studies of internal anatomy; namely that of the acoustic hearing apparatus, of which many aspects remain a mystery (Sarria-S et al. 2017).

Another understudied characteristic of these species is their use of ultrasonic acoustic signals and associated hearing capabilities. While the bioacoustics of *Asiophlugis* spp. have been recently thoroughly described (Tan et al. 2019), only four other species of Phlugidini to date have been acoustically studied (*Phlugis ocraceovittata*, Chamorro-Rengifo and Braun 2016; 3 unidentified species, Suga 1996, Montealegre-Z et al. 2006), leaving a gap in our understanding of the hearing and singing capabilities of these species. These existing studies find the Phlugidini calling frequency range to be 40-60 kHz (Chamorro-Rengifo and Braun 2016; Tan et al. 2019). However, with the great diversity of the calling song frequency of neotropical bush-crickets, including some well above 100 kHz (Sarria-S et al. 2014), it is plausible that the observed range of Phlugidini calling frequencies is an incomplete assessment of their true capabilities.

Using a combination of image processing techniques, Laser Doppler Vibrometry (LDV), and signal processing techniques, we report on cuticle transparency, biophysical analysis, and bioacoustic capabilities of a hereby newly described a monotypic Phlugidini genus from the Andean cloud forests of Ecuador, with exceptionally transparent species.

Methods

Study site

The Santa Lucia cloud forest reserve lies in the northwest of the Province of Pichincha in the Choco-Andean corridor; a protected region of tropical montane forest and a migration route for endemic and visiting species of Latin America. The reserve has an altitudinal range of 1300-2500 m, covering lowland and lower montane formations (Grubb and Whitmore 1966), and has a central lodge at 1920 m (0° 7' 20.3592'' N 78° 40' 3.3456'' W, GPS-Garmin eTrex 30). The environment is mixed, with primary and secondary forest as well as reforested areas and pastures for mules and cattle. Access through the reserve is limited to a series of footpaths.

Specimens

Specimens were collected from the field during July 8-17, 2018 and February 20-21, 2019, under permit N° 009 - 2018 - IC - FAU - DPAP – (Ministry of Environment). They were kept at the University of Lincoln, UK, in a communal tank in a PHCBI MIR-154 cooled incubator, with a 6-step 24-hour temperature cycle around a mean of 16.5°C. They were fed a diet of drosophila, cut apple, fish flakes (Love Fish, Wilmslow, UK), and water, and had access to a substrate and bromeliads similar to those in their natural environment.

Depository**:** MEUCE = Museo de Entomología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

Field Recordings

Field recordings (N=4) were taken using an Echo Meter Touch 2 (Wildlife Acoustics Inc., Maynard, USA) at a sampling frequency of 256 kHz. The distance of the recording device from the specimen was ~30 cm.

Lab Recordings

In the lab, calls were acquired using a 1/8 ” microphone (Brüel & Kjær, Nærum, Denmark) connected via a nexus amplifier (Brüel & Kjær, Nærum, Denmark) to PSV acquisition software (Polytec GmbH, Waldbronn, Germany). A high pass filter was set at 1 kHz, with a sample frequency of 256 kHz. Males (N=3) were placed in a fabric mesh net 20 cm from the microphone, with a female nearby to induce a call. The temperature in the recording booth was 18 °C and relative humidity ~40 %. For every call recorded, each chirp was individually extracted for analysis (N=94). The Power Spectral Density (PSD) of all chirps was calculated and averaged using MATLAB 2019 (MathWorks, Natick, USA).

Depository of sound recordings**:** IAVH = Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá d.c., Colombia.

Forewing resonance

Resonance of the tegmina was measured in 4 male specimens of *S. hishquten* using micro-scanning LDV (PSV-500, Polytec GmbH, Waldronn, Germany), with approximately 1750 grid points at a sampling frequency of 256 kHz. Wings were extended using a wax made of 50 % beeswax (Fisher Scientific, Loughborough, UK) and 50 % colophonium (Sigma-Aldrich Company Ltd., Dorset, UK). For full details of mount, see Sarria-S et al (2016). Acoustic signals for wing excitation were generated by a PSV-500 internal data acquisition board (PCI-4451; National Instruments, Austin, TX, USA), and consisted of broadband periodic chirps ranging from 2-80 kHz. These were then amplified (A-400, Pioneer, Kawasaki, Japan) and transmitted to a loudspeaker (Ultrasonic Dynamic Speaker Vifa,

Avisoft Bioacoustics, Glienicke, Germany) positioned 30 cm in front of the specimen. The amplitude of this stimulus was mathematically corrected, in situ, and delivered at 60 dB (SPL). The reference signal was recorded using a 1/8 ” condenser microphone positioned horizontally at the wing plane between the wings (Brüel & Kjaer, 4138-A015 and preamplifier model 2670, Brüel & Kjaer, Nærum, Denmark).

Cuticle transparency

Cuticle transparency of females (N=2) and males (N=4) was quantified by measuring the transmittance (ratio of the transmitted radiant flux to the incident radiant flux) of the cuticle covering the hearing organs. We measured the transmittance of the LDV light beam (633 nm, PSV-500, Polytec GmbH, Waldronn, Germany) as a reference. For full details of the method, see Sarria-S et al (2017).

Morphological measurements

Measurements of the general morphology of specimens was carried out using a digital calliper under a low power microscope. The whole-body measurement is a measure from the frons to the end of the last tergite. The male subgenital plate and female ovipositor were excluded in the whole-body measurement. Tooth length and inter-tooth distances of the stridulatory file were measured using the scale bar provided with images produced by an Alicona InfiniteFocus microscope (Bruker Alicona, Graz, Austria).

Results

Taxonomy

Speculophlugis Woodrow et al. n. gen.

Etymology

This name is composed of the Latin speculo, meaning glass or 'looking glass', due to the exceptional level of transparency compared to other Phlugidini genera, allowing us to observe internal anatomy; and *Phlugis*, named after the type genus of the tribe Phlugidini

Type-species

Speculophlugis hishquten, here described.

Diagnosis

Several characters separate *Speculophlugis* from other Phlugidini genera. General morphology very similar to *Lucienola* (Rentz, 2011) but differs significantly by geographic location. Genitalia most similar to *Phlugiola* (Karny, 1907), with styli not as elongate. Mandibles display sclerotization at apex with slight asymmetries present in the width of mandibles (Fig. 2A) but not as extreme as in *Anisophlugis* (Chamorro-Rengifo & Olivier, 2017). Pronotum comparatively reduced and unspecialised for brachypterous Phlugidini.~~,~~ Key diagnostic features include male tegmina; which are unusually humped for Phlugidini (Fig. 3A), with hindwings present but heavily reduced, and cuticular transparency; which is clearly observable in live specimens (Fig. 1) and significantly greater than other observed Phlugidini species.

Speculophlugis hishquten sp. nov. [Figure 1 near here]

Holotype

MEUCE 1♂ Ecuador, Pichincha, Nanegal, Reserva Santa Lucía. Collectors: C. Woodrow, C. Soulsbury, F. Montealegre-Z., July 8-17, 2018.

Allotype

MEUCE 1♀ Ecuador, Pichincha, Nanegal, Reserva Santa Lucía. Collectors: C. Woodrow, C. Soulsbury, F. Montealegre-Z., July 8-17, 2018.

Paratypes

MEUCE 3♂2♀ Ecuador, Pichincha, Nanegal, Reserva Santa Lucía. Collectors: C. Woodrow, C. Soulsbury, F. Montealegre-Z, July 8-17, 2018.

Etymology

This species is named *S. hishquten*, a reference to the fictional extra-terrestrial character Hish-Qu-Ten featured in the Predator franchise (McTeirnan et al.1987). This is due to several comparable features: tropical climate habitat; transparency of *S. hishquten*'s cuticle reflecting the ability of Hish-Qu-Ten to become invisible; the vibrant chartreuse green of *S. hishquten* matching Hish-Qu-Ten's glowing green blood; and *S. hishquten*'s stealthy hunting technique.

Description

Head. Arrow-shaped, narrow (Fig 2A). Eyes prominent and ellipsoidal. Frons narrow, small. Most of the head dominated laterally by gena, and distally by ridged clypeus and narrow convex labrum. Maxillary palps large for the genus, mandibles thin, extensible.

Thorax. Pronotum flat for Phlugidini, 1.8 to 2.0x longer than wide, anterior and posterior margin convex but anterior margin more squared in shape (Fig. 2D). Pronotal disk campaniform but reduced. Prozona elongate.

Legs. Fore femora with four spines per inner ventral margin, reducing in size proximally. Fore tibiae with five movable spines per inner ventral margin, reducing in size proximally and distally from centre spine. Both fore femora and fore tibiae spines evenly spaced and facing forwards. Fore coxal spine sharp, facing backwards. Hind femora much larger at base, tapering distally (Fig.2F). Anterior and posterior tympana present, naked, and large for leg size (Fig. 2B). [Figure 2 near here]

Wings. Brachypterous. Male tegmina humped with structures for sound production facing more anterior than other genera. Male tegmen to pronotum ratio 1.15:1 (N=8). Female tegmen to pronotum ratio 0.85:1 (N=6). Hindwings present but very heavily reduced, less than 0.5mm long in both sexes. Stridulatory file 0.51 ± 0.02 mm long bearing 27-29 teeth (Fig. 3C). Tooth length varies irregularly but tends to be greatest around centre of file, and inter-tooth distances display no distinct pattern along the file (Fig. 3C). Stridulatory file most similar to that of *Phlugis chyrsopa* (Nicke 2003). [Figure 3 near here]

Abdomen. Tenth tergite produced distally and bilobular in males (Fig.2E). Male cerci incurved with flat sclerotized tip and dense hairs (Fig. 2E). Female cerci straight and pointed with sclerotized tip, and around 3.0x shorter than ovipositor.

Genitalia. Male Subgenital plate bilobular with incurved styli; one per lobe (Fig. 2E). Female subgenital plate pointed and spatulate. Ovipositor large, curved and around 3.0x longer than cerci (Fig. 2C).

Variation. *Eyes* opaque lime green with variation in brown markings *in vivo*. Individual variation in depth of chartreuse green colour of body, and in fragmentation of dorsal line of dark pigmentation.

Colouration. Uniform chartreuse green *in vivo*, yellow to light green in preserved specimens. Dark pigmentation strip running posteriorly from each compound eye, and a single dorsal line of dark pigmentation running from posterior margin of pronotum to tenth tergite (Fig. 1). Dark pigmentation pattern maintained in preserved specimens. Eyes bright chartreuse green *in vivo*.

Measurements. See Table 1. [Table 1 near here]

Behavioural ecology. In the field, *S. hishquten* is well camouflaged and difficult to initially detect. When observed, male individuals would rest on the underside of leaves of the family Marantaceae. The reason for this preference is unknown. Once prey landed on the surface of the same leaf, the male appears to stalk the prey shadow to the very edge of the leaf, before moving around the edge of the leaf and striking. In the lab, we have observed both *S. hishquten* and *Phlugis poecila* (Hebard, 1927) occasionally exhibiting the same behaviour if an artificial leaf is provided. The same behaviour has been reported before in the Suriname species *Phlugis teres* (Naskrecki 2013). Individuals in the lab and field also frequently elicited a lateral motion, with the abdomen acting as a pivot, prior to jumping. This behaviour is often associated with depth perception by motion parallax (Collett, 1978) and suggests that in *S. hishquten*, vision plays a great role in environmental assessment and prey detection. This species exhibits purely diurnal calling.

S. hishquten displays a rapid-decay pulse and repeat song structure, with 8-12 calls per second (Fig. 4A). The average duration of one song was 10 ± 2 s. Each call within the song consists of either 2 or 3 syllables, with random prevalence (Fig. 4B). The major pulse of each call contains 8-10 oscillations (Fig. 4C). Each pulse is ~16.1 ± 4.7 ms in duration. The average downtime between consecutive pulses is 98.2 ± 22.8 ms. At -30 dB, spectral breadth ranged from 31.9-73.2 kHz. Welch's PSD analysis of 94 calls revealed a fundamental frequency of 50 kHz and mean peak frequency of 49.9 ± 2.2 kHz (Fig. 4E). The spectral breadth of a single pulse measured at -30 dB below highest energy peak spread to 30-40 kHz (Fig. 4D), reflecting the relative broadband nature of this call.

[Figure 4 near here]

Wing resonance

Surface LDV scans provided resonance of both wings for 4 male specimens. At 50 kHz, the fundamental frequency of the male song, both wings displayed vibration resonance in antiphase (Fig. 5A). The maximum velocity was greater in the right wing (52 µ/s, Fig. 4B) than the left wing (32 µm/s, Fig. 5C). The right wing also displayed a peak vibration resonance at 52 ± 1.5 kHz, while the left displayed a peak vibration resonance at 45 ± 2 kHz (Fig. 5D), both within the carrier frequency of the conspecific song.

[Figure 5 near here]

Cuticle Transparency

Transmittance percentage values for all measured cuticles at the dorsal ear cuticle (two males and two females) increased with wavelength in the visible light spectrum, 370–800 nm (Fig. 6). At the light spectrum wavelength of the LDV beam, the transmission values were very high, at 85% and 87% for females and males respectively (Fig. 6) Additionally, the cuticle of the hind femora was measured in one male individual, showing transmittance of 94%.

[Figure 6 near here]

Discussion

Acoustics and species-specific markers

There are 17 valid genera described in the tribe Phlugidini (Cigliano et al. 2019). *S. hishquten* is described as a new monotypic genus to science based on morphological evidence. The male call of *S. hishquten* is consistent with the frequency range of existing Phlugidini species, which all show ultrasonic calling frequencies of 40-60 kHz (Tan et al. 2019; Chamorro-Rengifo and Braun 2016). In *S. hishquten*, a repetition rate range of 8-12 calls per second was observed across field conditions (~18 °C) and lab conditions (~22 °C, 40 % relative humidity), with 2-3 discrete syllables per call. The variation between lab and field recordings is likely a result of differences between temperature and relative humidity, yet the unpredictable prevalence of both 2-pulse calls and 3-pulse calls was exhibited both in the lab and the field. The call of *S. hishquten* is most like that of *Asiophlugis temasek tioman* in waveform structure (Tan et al. 2019). *S. hishquten* displays a broad carrier frequency spectrum, with a spectral width of 31.9-73.2 kHz at -30 dB, while *A. temasek tioman* displays a carrier frequency spectrum of ~45-60 kHz. This broad spectral width of Phlugidini calls likely results from contrasting wing resonances and stridulatory file morphologies.

In *Asiophlugis*, a broader coverage of species has allowed for a greater assessment of call structure variation and has demonstrated how to assess and compare acoustic characters of Phlugidini species. Interestingly, song call variation between allopatric and sympatric *Asiophlugis* species does not differ as would be expected for species sharing the same communication channel of 40-60 kHz (Izzo and Gray 2004; Tan et al. 2019). However without the discovery and song recordings of more *Speculophlugis* species, we are unable to confirm whether the same observation applies to this genus. Our results and the above-

mentioned articles suggest that Phlugidini species have evolved ultrasonic calls with a time domain structure comprised of discrete short pulses. These pulses are made of a few oscillations (8-10 in this case) and appear to be produced by scraper springing mechanism. In other ultrasonic species (*Supersonus*, Sarria-S et al. 2014), the scraper is paused behind a file tooth, then as the wings exert pressure against each other the scraper is deformed and released by elasticity, springing forward over a few teeth, producing short pulses that reflect the number of teeth struck (Montealegre-Z et al. 2006). *Speculophlugis*, as other high ultrasonic insects, are very fragile and thin, and muscular mass is likely to be too small to propel the wings at high speed, therefore elastic energy may be their best tactic to strike several file teeth at high velocity.

Existing comparisons suggests that stridulatory file morphology is a useful character in differentiating Phlugidini species but not subspecies (Nicke 2003; Tan et al. 2019). This allows us to further justify describing *Speculophlugis* as a new genus due to its much shorter (~29 teeth) and uniquely curved file, which is unique among recorded neotropical species, which display tooth number variation of ~37-82 (Nicke 2003, 2005).

Wing resonances

The wings of crickets, grigs and katydids can be regarded as dipole sources, which is equivalent to a pair of sound sources of opposite sign situated close to one another, with resultant sound leakage from one surface to the other (Bennet-Clark 1998). While sound production in crickets and grigs involves the compliance of both tegmina which are nearly mirror images of each other (Montealegre-Z et al. 2011, Chivers et al. 2017), sound radiation in many katydids is achieved using strongly asymmetric tegmina. In such species the right tegmen radiates most of the acoustic energy involved in the call output, and the left tegmen is usually damped (Montealegre-Z & Postles 2010; Sarria-S et al. 2014; Heller & Hemp 2014;

Sarria-S et al. 2016; Baker et al. 2017, Chivers et al. 2017). In some species like *Ectomoptera nepicauda* (Ragge 1980) this asymmetry is such that the left tegmen carries only the file and the mirror areas have completely disappeared (Heller & Hemp 2014). Two working hypotheses have been proposed to explain this asymmetry (Montealegre-Z 2005). One suggests that symmetric radiators work as two synchronised speakers to maximise the area of sound radiation (which for dipole sources as the Ensifera wing should be about ¼ of the wavelength, Montealegre-Z 2005) while maintaining the purity of the call that enhances directional hearing, for example in field crickets. But at high frequencies, synchronisation of the 'two speakers' could be more challenging, and the evolutionary solution could be the acoustic damping of the file-bearing wing (left tegmen), and radiate sound with one sharply-tuned wing to maintain purity at high frequencies. The consequence of this is the reduction of sound radiating area, and these katydids might have other strategies to increase loudness (Bennet-Clark, 1998), for example critical positions of the wings (Montealegre-Z & Mason 2005), transforming a dipole system into a monopole radiation (Sarria-S et al 2014), or resonant cavities (Jonsson et al. 2017). The Phlugidini tribe includes gracious species with small body size and minute wings with small sound-radiating areas. These insects might be too small to afford sound radiation with a single small wing and having two active radiators might help them to overcome the problem of being small and loud at the same time. Although the wings contribute to sound radiation differently (i.e., with different amplitudes of deflection, and different resonances, Fig. 5B-D), the area gained by the two radiating wing cells could be optimal to radiate a wavelength of about 7 mm at the calling song frequency. The combined resonances at about 45 kHz for the left tegmen, and 52 kHz for the right tegmen, might also explain the broadband nature of the call spectrum in this species (Fig. 4D). Two functional but asymmetric wing radiators have been observed in other katydids, for example in the genus *Afroanthracites*, resulting in broadband calls (Hemp et al. 2015).

Transparency, Ecology and Behaviour

The transmittance of the cuticle of *S. hishquten* (85-87 %) measured at the LDV wavelength using standardised methods is the most transparent bush-cricket cuticle measured to date. One species of the same tribe (*P. poecila*) was reported to show a lower cuticle transmittance of 73-74 %; a feature that facilitates non-invasive measurements of auditory process in the inner ear (Sarria-S et al. 2017). Our results here suggest that *S. hishquten* has great potential to be a model species for non-invasive hearing research, due to this attribute.

Terrestrial transparency is a rare occurrence in nature. This is hypothesized to be, in part, due to a large difference in the refractive index of organismal tissues compared to that of air; with the surface reflection of a transparent organism in air being 10-fold to 2000-fold greater than that of a transparent organism in water (Johnsen 2001). A higher surface reflection of terrestrial organisms means that transparency is not an effective method of camouflage in most terrestrial habitats, and so is rarely favoured by natural selection. Experimental manipulation of water salinity and temperature has shown to induce tissue transparency in glass shrimp (Bhandiwad and Johnsen 2011), furthermore suggesting that variation in the refraction index of the medium in which an organism exists alters the efficiency, and therefore the selection, of transparency. For *S. hishquten*, we hypothesise that an ultrasonic song combined with high transparency allows for the exploitation of a niche uncommonly fulfilled by tropical bush-crickets – diurnal activity. By performing high frequency calls, *S. hishquten* is much less likely to be predated due to potential restrictions of the hearing capabilities of predators. Furthermore, the transparency and green pigmentation of the cuticle greatly match the patterning and light refraction of water droplets on a leaf (Fig. 1), which could act as a diurnal defence against vertebrates by simple passive crypsis (Johnsen 2001).

With their ultrasonic communication, unique hunting ecology, and high cuticular transparency, we believe *S. hishquten* to be of unique potential as a model for assessments of ultrasonic evolution, non-invasive internal anatomy, and predatory katydid ecology.

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Author contributions

CW conducted fieldwork, analysed data, wrote the paper, named and diagnosed the genus and species, and prepared figures. CP conducted fieldwork, helped with literature review and writing. DV conducted fieldwork, obtained sound recordings, helped with taxonomic search and made helpful comments to manuscript. FMZ helped to diagnose new species, conducted fieldwork, obtained field and lab recordings, helped with writing and led and provided argument for research.

Disclosure statement

There are no conflicts of interest between authors

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Table 1. Morphological measurements of *Phlugis Hishquten*. All measurements in mm. F, fore; H, hind; M, mid; SD, standard deviation.

Character	Males (N=5)	SD	Females (N=6)	SD
Body	11.78	0.40	13.00	0.60
Tegmen	3.03	0.17	2.66	0.07
Pronotum length	2.81	0.21	2.62	0.26
Pronotum width	1.43	0.04	1.43	0.11
Subgenital plate	0.89	0.04	0.82	0.07
Stridulatory file	0.51	0.02	n/a	n/a
F-Femur	4.41	0.83	4.61	0.23
F-Tibia	5.26	0.61	5.18	0.37
M-Femur	5.42	0.20	4.92	0.23
M-Tibia	5.78	0.01	5.64	0.19
H-Femur	11.16	0.23	10.95	0.53
H-Tibia	11.79	0.23	11.51	0.48
Eye length	1.12	0.05	1.12	0.09
Eye width	0.94	0.05	0.84	0.07
Eye depth	0.62	0.01	0.70	0.06
Cercus	1.03	0.03	1.41	0.10
Ovipositor	n/a	n/a	4.09	0.69

Figure legends:

Figure 1. *Speculophlugis hishquten* sp. nov. Male colour and patterning across the dorsal surface, and observable transparency. Refraction of light through the cuticle, particularly in the hind femora, appears to mimic that of a water droplet.

Figure 2. Anatomical features of *Speculophlugis hishquten* sp. nov. A, face; B, left leg anterior tympanum; C, ovipositor; D, pronotum; E, male genitalia in (i) dorsal, (ii) ventral, (iii) lateral, and (iv) frontal view; F, full female specimen, showing the great size of ovipositor. Images produced by Alicona InfiniteFocus. Illustration by CW.

Figure 3. Tegmina and stridulatory file anatomy. A, Lateral view of male wings, showing hump shape; B, Stridulatory file area on left tegmen; C, Tooth distribution across the length of the file, based on tooth length (filled, black circles) and inter-tooth distances (open, red circles).

Figure 4. Description of male calling song under laboratory conditions. A, section of song consisting of 6 calls with B highlighted; B, high resolution of a single 2-syllable call with C highlighted; C, high resolution of single major pulse; D, frequency spectrum of a single call pulse; E, Welch's Power Spectral Density analysis of averaged calls (N=94), with values of the first 3 peak frequencies given.

Figure 5. Wing resonances. A, wing velocity at 50 kHz phase 180 °, showing transect lines for B and C; B, velocity along right wing transect at 50 kHz, for phase intervals of 15 °; C, velocity along left wing transect at 50 kHz, for phase intervals of 15 °; D, Both wing resonance spectra.

Figure 6. Transmittance curves (percentage of light diffused through the ear dorsal cuticle) measured in the visible light spectrum (370– 800 nm). At the laser beam wavelength (633 nm, dotted line) cuticle transmittance was 85-87 % for females and males respectively (n♂=4, n♀=2). Maximum observed transparency of 94 % was observed in the cuticle of the hind femora of one male individual, curve not shown here.

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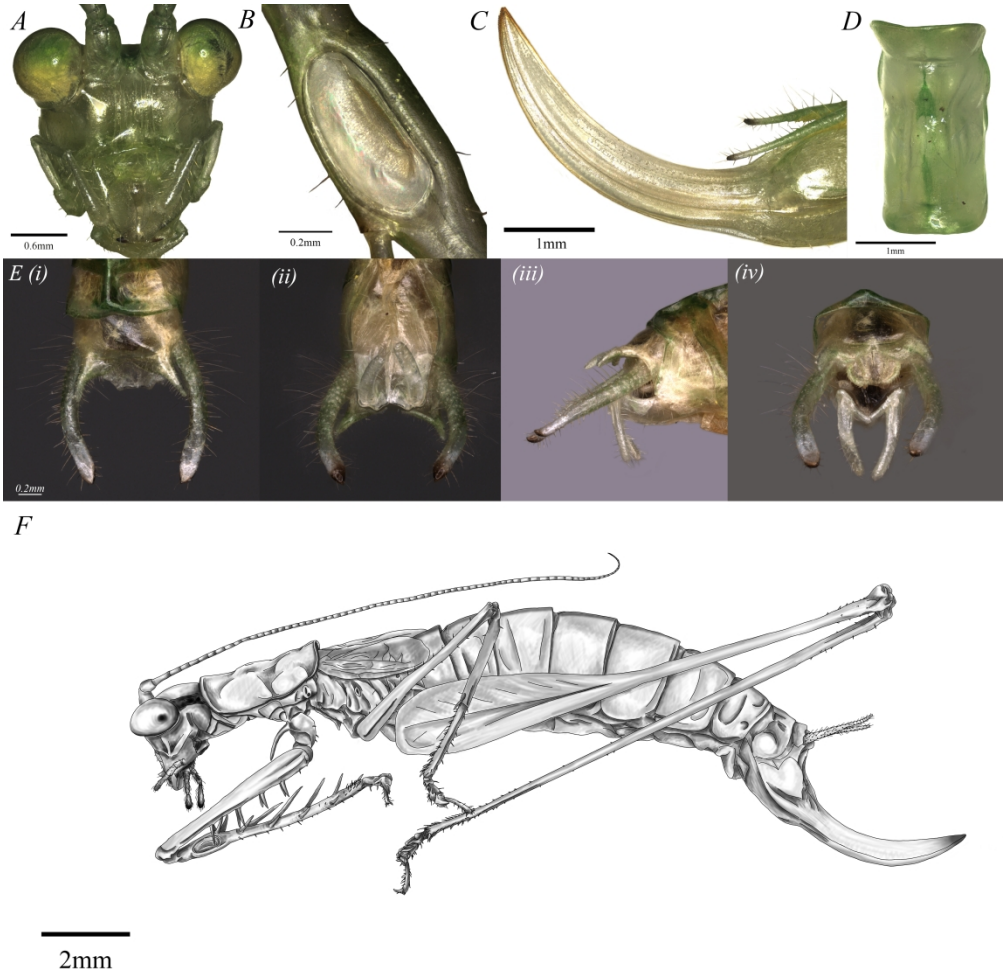

Figure 2. Anatomical features of Speculophlugis hishquten sp. nov. A, face; B, left leg anterior tympanum; C, ovipositor; D, pronotum; E, male genitalia in (i) dorsal, (ii) ventral, (iii) lateral, and (iv) frontal view; F, full female specimen, showing the great size of ovipositor. Images produced by Alicona InfiniteFocus. Illustration by CW.

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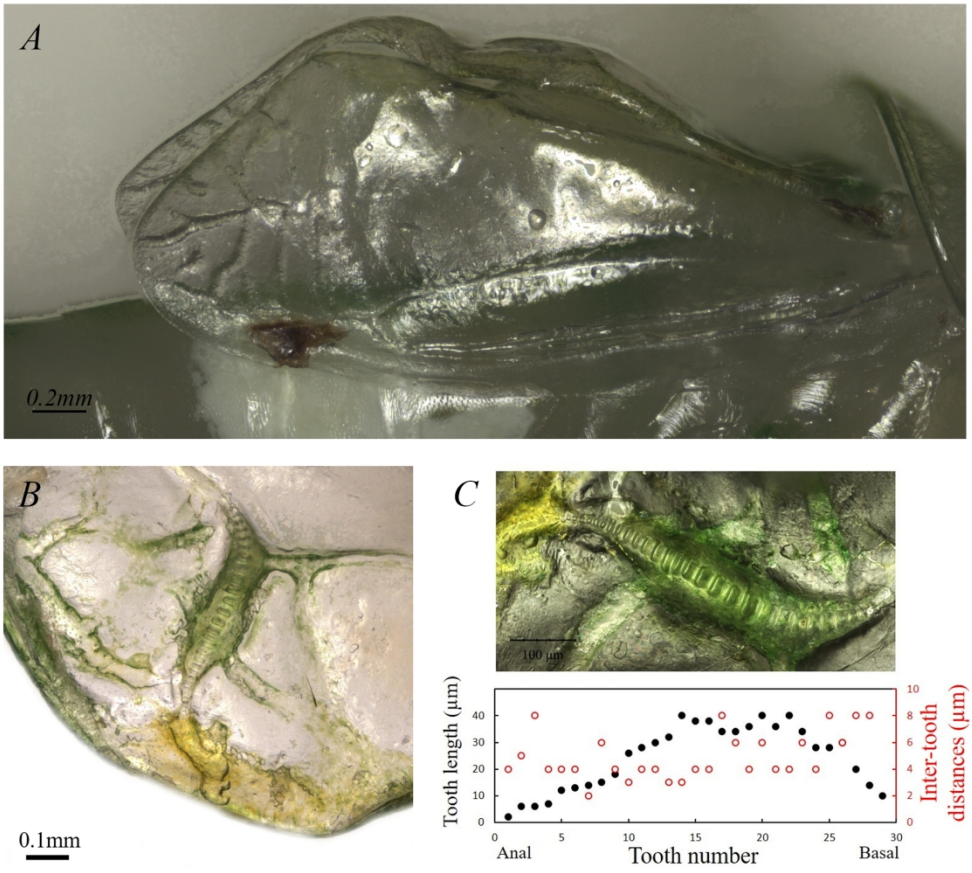

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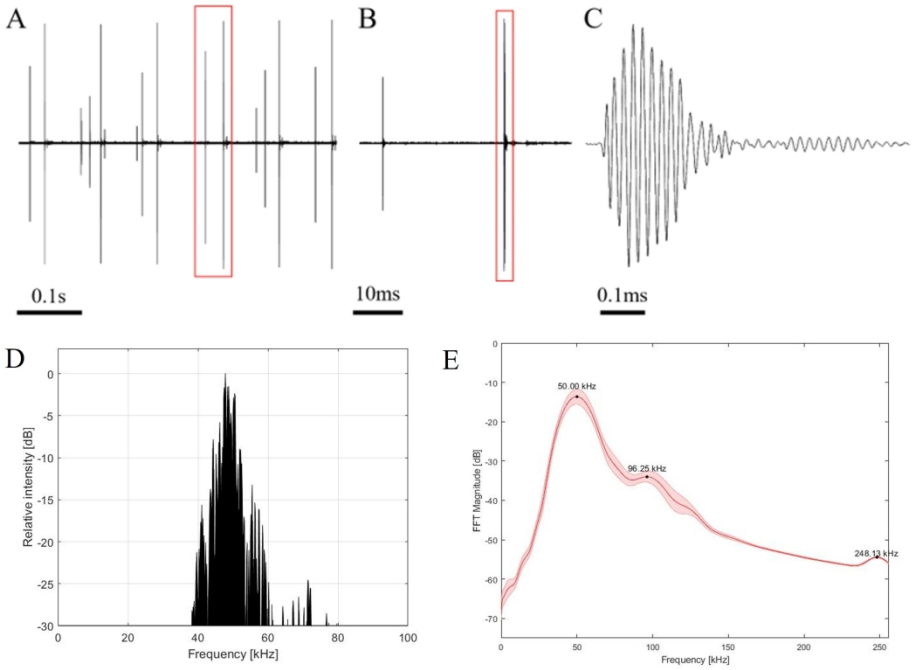

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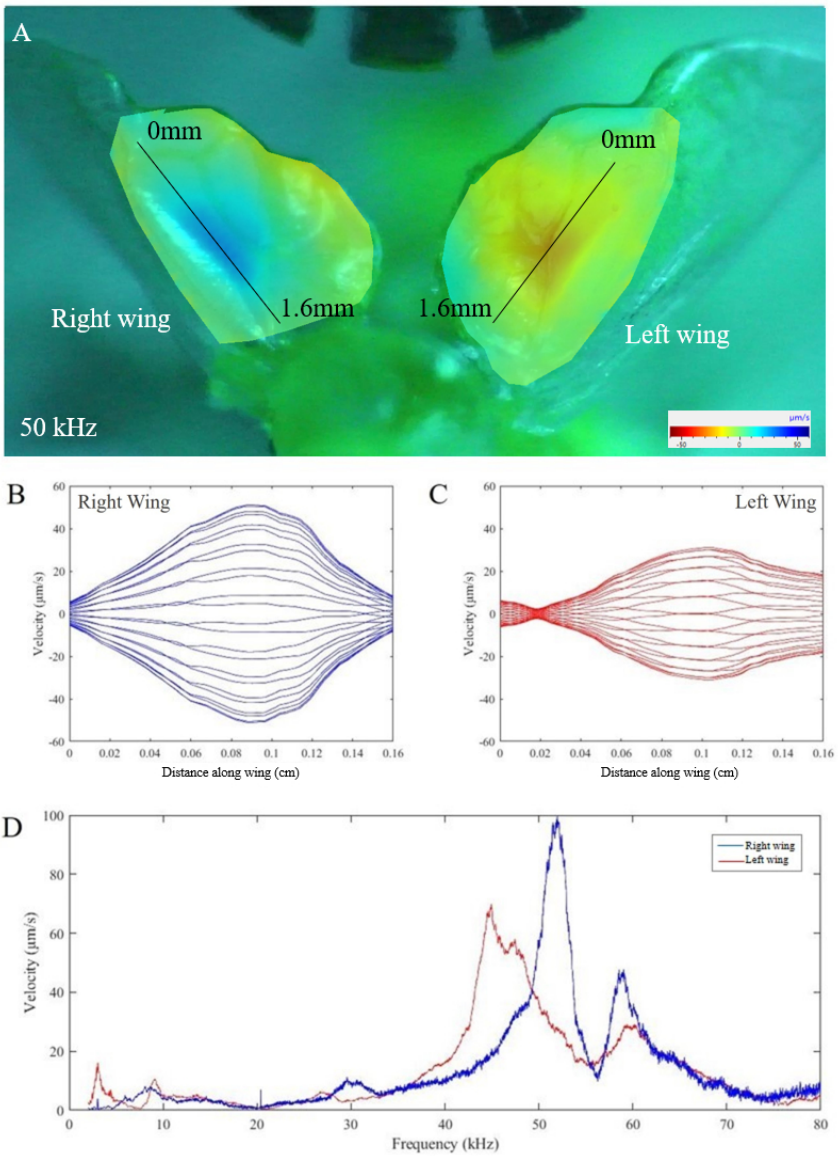

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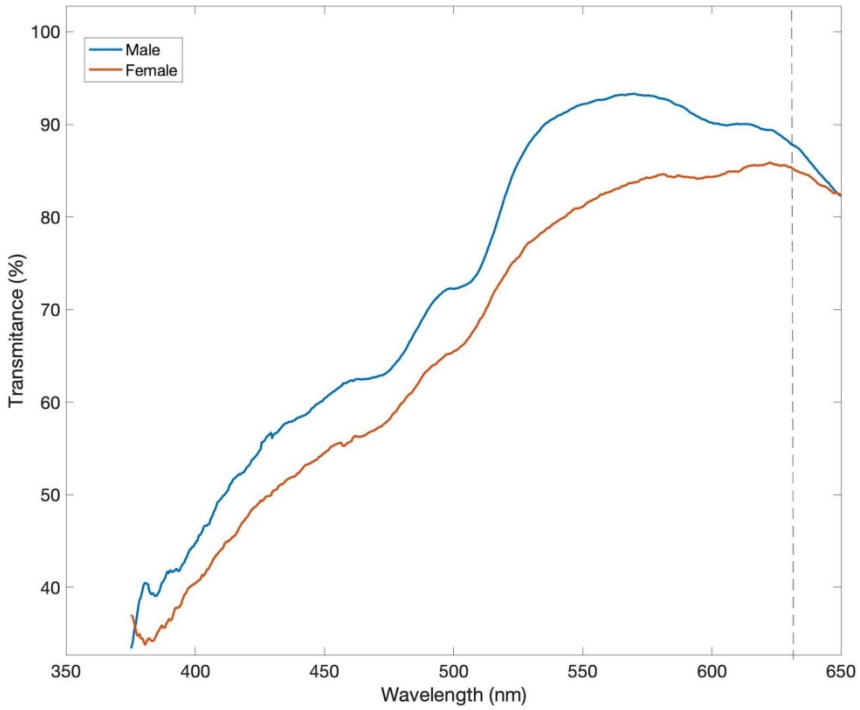

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299x238mm (300 x 300 DPI)